IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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SERIAL NO.: 10/521,922 ART UNIT: 1645

FILED: August 29, 2005 EXAMINER: Gangle, B. J.

TITLE: SPECIFIC ISOTYPE ANTIBODIES OF SECRETION-EXCRETION ANTI-ANTIGENS OF *LEISHMANIA SP* OF PROMASTIGOTE OR AMASTIGOTE FORMS, etc...

Amendment B: REMARKS

Upon entry of the present amendments, Claims 1-15 have been canceled and Claims 16-21 have been substituted therefor. Reconsideration of the rejections, in light of the forgoing amendments and present remarks, is respectfully requested. The present amendments have been entered for the purpose of more clearly distinguishing the present invention from the prior art and for the purpose of placing the claims into a condition for allowance.

In the Final Action, it was indicated that Claims 1-9 were previously canceled and that Claims 12-15 were withdrawn as being drawn to a non-elected invention.

Claims 10 and 11 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement and as failing to comply with the enablement requirement. Claims 10 and 11 were rejected under 35 U.S.C. § 102(b) as being anticipated by the Deplazes reference. Claims 10 and 11 were also rejected under 35 U.S.C. § 102(b) as being anticipated by the Afrin reference. Claims 10 and 11 were further rejected under 35 U.S.C. § 102(b) as being anticipated by the Sartori reference. Claims 10-11 were also rejected under 35 U.S.C. §112, second paragraph for indefiniteness. Objections to the specification have also been made with respect to formalities.

As an overview to the present reply, Applicant has re-presented the previously amended claims from the direct French translation submitted in the original filing, including changes to address the rejections and objections of the Examiner. The proper claim formalities in the method claims, Claims 18-21, are not properly dependent on independent Claim 16. The composition claims have been presented as Claims 16-17, corresponding to Claims 10-11. The method claims have been re-presented as Claims 18-21, incorporating the proper language to associate the composition of Claim 16 with the method claims. Applicant has also amended the specification to correct the formalities objected to by the Examiner.

In reply to the Office Action, Applicant has extensively amended the independent claim so as to more accurately claim the composition of the present invention. The independent composition claim now incorporates the subject matter of original Claims 2-4. Applicant has entered these amendments in response to the rejections under 35 U.S.C. §112, first and second paragraphs. The claim language is no longer drawn to a product of nature, and the limitations now sufficiently describe and define the subject matter of the composition.

In further reply to the 35 U.S.C. §112, first and second paragraph rejections, Applicant's attorney has been provided with a response from the inventors.

First of all, the inventors respectfully note that the overall aim of the invention disclosed in the present patent application is to offer to veterinarians a test allowing the distinction between infected dogs and immunized dogs.

The infected dogs show total IgG (antibody) specific to total antigen of *Leishmania infantum* and also IgG_2 to this total antigen of *Leishmania infantum*.

However, all these infected dogs, symptomatic and asymptomatic, generate these antibodies,

but the infected dogs do not generate any IgG_2 specific to the carboxyterminal part of the PSA protein. The PSA protein is a major antigen of the antigens excreted-secreted at the two life cycle stages of *Leishmania sp.* : amastigotes and promastigotes.

Only dogs immunized with the vaccine complex previously developed by the inventors and disclosed in French patent N°01/07606 (already cited herein with a corresponding U.S. application as U.S. Serial No. 10/480026) are able to generate specific IgG_2 to the carboxyterminal part of the PSA protein. Such a test is essential to distinguish infected dogs from immunized dogs, especially following a level of specific IgG_2 for a potential boost.

The inventors further contend that when they filed for patent protection initially in France in 2002, they began the research and development on the carboxyterminal part (C-ter) of the PSA protein. At this time, they developed the first generation vaccine against leishmaniasis in dogs corresponding to the above-mentioned French patent N°01/07606, and its corresponding U.S. Serial No. 10/480026. Since 2002, the inventors have continued to carry out several experiments on leishmaniasis, especially the development of a second generation of vaccine based on C-ter of PSA.

In response to the rejections for lack of written description and enablement, the inventors respectfully contend that "cryptic or immunologically silent epitope" is sufficiently known by one skilled in the art.

There are immunodominant epitopes recognized by antibodies and "subdominant" epitopes ("cryptic"according to the scientific community) which are silent until they were recognized or "unmasked ". The dominance is that some potential epitopes, during an immune response, are neglected in favor of others against whom the bulk of the immune response will be put into place. In the present invention, the epitope, located in the carboxyterminal part of the PSA, is silent in case

of a natural infection, but it is "unmasked" and activated in the context of an immunization by the vaccine complex disclosed in the related patent application according to the previously referenced U.S. Serial No. 10/480026.

A vaccine consists of an antigen and an adjuvant, the adjuvant being used to potentiate immune responses against an antigen. The adjuvant is described in French Patent No. 01/07606, corresponding to U.S. Serial No. 10/480026, and preferably induces a cell-mediated response. This implies that the epitopes located in the carboxyterminal part of the PSA be hidden (silent) in case of a natural infection, but when the carboxyterminal part of the PSA is injected to dogs in presence of such an adjuvant, said epitopes are unmasked and therefore antibodies specific to these epitopes are produced.

So the epitope located in the carboxyterminal part of the PSA and mentioned in the application is silent in case of natural infection, but is unmasked in immunized dogs. The presentation to the immune system of antigen by the parasite may be different from that of soluble antibodies injected in the presence of an adjuvant.

Additionally, the term, "full characterization of the antibody IgG_2, the enitope, and the carboxyterminal part of the PSA" satisfies the written description not enablement requirement. With reference to Figures 1-3 of the priority document, the carboxyterminal part of the PSA is disclosed. In particular, in FIGURE 1, the Protein Surface Antigens are identified by name: A3B, 1A1, W2 and 2G1. Their carboxyterminal parts (C-terminal) are identical to the sequence B3A. FIGURE 1 is a schematic representation of different proteins translated from different clones of cDNA isolated during a screening of cDNA bank of promastigotes and amastigotes of *L.infantum*. The proteins A3B, 1A1, W2 and 2G1 differ among themselves by a different number (4 to 7) of patterns rich in

Leucine (LRR). B3A represents a truncated protein issued from a clone of cDNA corresponding to the carboxyterminal part of the PSA (C-ter). B3A could be produced recombinantly in *E. coli* and be used as an antigenic substrate in Figures 2 and 3.

FIGURE 2 describes the IgG_2 response against the C-ter (recombinant) part of the PSA in patients suffering from leishmaniasis, there being different leishmaniasis. For example, *L.infantum* and *L.chagasi* provoke visceral Leishmania, and *L.amazonensis* causes a cutaneous leishmaniasis. FIGURE 2 also shows the response in dogs naturally infected by *L. infantum*. No response is observed. So there is no production of antibodies anti-C-ter (recombinant) during an infection.

FIGURE 3 describes the same response from the party C-ter of recombinant PSA in dogs vaccinated. These dogs produce antibodies IgG_2 anti-C-ter of recombinant PSA (positive Western blot). Thus, the infected dogs can be distinguished from the vaccinated dogs.

The inventors respectfully contend that, as shown in Figure 2, patients and infected dogs do not produce antibodies of isotype IgG_2 against the carboxyterminal part of the PSA, unlike vaccinated dogs, as shown in Figure 3. The vaccinated dogs show the presence of antibodies of isotype IgG_2 against truncated recombinant protein corresponding to the C-ter part of the PSA. The antibody F5 is a monoclonal antibody directed against a specific epitope of the C-ter part of the PSA and used in these drawings as a control.

With regard to the description and definiteness of the efficacy of the immunoglobulins IgG_2 to lyse amastigotes and promastigotes of *Leishmania sp.*, the inventors respectfully contend that the recognition of the epitope by the IgG_2 releases the complement cascade, which leads to lysis the cell. The IgG_2 does not have the capability to directly lyse the cell. The general principles of the mechanisms of cell-mediated immune response are described in the more conventional manuals of

students. One skilled in the art can be familiar with the now proper phrasing as triggering the immune response, resulting in eventual lysis of the cell.

Relative to the prior art, claims of the present application are limited to dogs. Furthermore, as previously explained, the cited documents concern only animals infected by Leishmania sp. and not immunized against it. So there are not capable of inducing an immune response specific to the carboxyterminal part of the PSA.

In response to the rejections based upon prior art, Applicant notes that the Deplazes reference, Afrin reference and Sartori reference disclose IgG2 antibodies in dogs infected with L. infantum, in mouse immunized with antigens of L. donovani, and in hamster infected with L. donovani, respectively. The present invention is a composition of IgG2 antibodies specific to the carboxyterminal part of an antigen in dogs immunized with antigens of the promastigote forms and amastigote forms of L. infantum. Furthermore, the prior art references are not distinguishing between infected dogs and immunized dogs. The different characteristics of the immunized dogs are not anticipated by the prior art compositions. Thus, the composition of the present invention is not disclosed by the proteins of the Deplazes reference and the Sartori reference. Those prior art references relate to only the IgG proteins of naturally leishmanian animals (see paragraphs 12 to 18). Similarly, the Afrin reference describes the humoral and cell-mediated immune responses in hamsters and mice, immunized with an antigenic extract of a parasite alone or encapsulated in liposomes. The present invention describes antibodies against the carboxyterminal part of an antigen not described in the Afrin reference. These antibodies of IgG2 isotype are selectively generated in dogs immunized with excretion-secretion antigens, of the Th1 type immune response. Thus, the antibodies of the invention have a specificity different of that those disclosed in the Afrin reference.

With regard to the method claims, the methods now include the proper references to the composition of Claim 16. The methods are specifically limited to the application and steps related to the claimed composition of immunoglobulins. The proper claim language format has attempted to remove the simple recitation of a use.

The specification has been amended to remove the references to drawings, and the correction of the Trypan blue has been corrected. It is important to note that the relevant drawings were submitted of record in the priority French application. A record copies of the priority document was transmitted to the PCT application. A copy of this document is attached hereto for reference. These figures are not presented in the present application as new matter. No new drawings are presented, since they have already been submitted in the application.

Applicant's U.S. attorney notes that a Phone Interview occurred on November 16, 2007 and November 23, 2007 by voicemail and direct contact. The substance of the interview included a discussion of the need for more specific claim language and possibility of another Examiner Interview with the inventors before the next Office Action.

Applicant's U.S. attorney respectfully requests a telephone call from the Examiner before the next Office Action. Applicant's U.S. attorney, Andrew W. Chu, can be contacted at 713-224-8080, ext. 206. Please have the Examiner contact the attorney when the application is ready for an interview. Advanced arrangements may needed for inventors in France, and there is a large time zone difference involved. Cooperation would be greatly appreciated.

Based upon the foregoing analysis, Applicant respectfully contends that independent Claim 16 is now in proper condition for allowance. Additionally, those claims which are dependent upon this independent claim should also be in condition for allowance. Reconsideration of the rejections

and allowance of the claims at an early date is earnestly solicited. Since no new claims have been added above those originally paid for, no additional fee is required.

A Request for Continued Examiner is filed concurrently herewith. The requisite fee is also included with this document.

A Petition for Extension of Time is filed concurrently herewith. The requisite fee is also included with this Petition.

Respectfully submitted,

December 12, 2007
Date

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Paragraph [0031] should be amended as follows:

[0031] A viability test with ~~TRYPAN BLUE (TM)~~ Trypan blue at 0.4% in PBS and a Thomas cell counting must be done in order to resuspend the parasites in sterile PBS so that they are at 10^6/ml.

Paragraph [0040] should be amended as follows:

[0040] One aliquot of 10 ul for each test is then taken immediately after contact in order to perform a viability test with ~~TRYPAN BLUE (TM)~~ Trypan blue and a Thomas cell counting, the surplus serving for the cultivation from which a counting is done daily.

Paragraph [0061] should be amended as follows:

[0061] Determined by a test with ~~TRYPAN BLUE (TM)~~ Trypan blue, the viability of the promastigotes was 100% before contact with the serum. After 30 min. of contact, the viability with the healthy serum was always 100% at all dilutions, while with the serum immunized with ESP, it was only more than 50% with the pure serum, 73% with the serum diluted to ½, 92% and 94% with respectively the serum diluted to ¼ and 1/8.